Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

STUDIES ON THE CULTIVATION OF THE VIRUS OF VACCINIA. II.*

EDNA STEINHARDT AND ROBERT A. LAMBERT.

(From the Research Laboratory, Department of Health, and the Department of Pathology, College of Physicians and Surgeons, New York City.)

Two years ago[1] we began to apply the method of cultivating tissues *in vitro* (Harrison) to the study of the viruses of certain infections in which the specific living agents have not yet been identified. The three viruses studied thus far with the new method have been those of rabies, vaccinia, and syphilis.[2] In the study of the former we attempted, first, to determine whether the Negri bodies, the nature of which is still in dispute, would show any development or multiplication *in vitro* when fragments of brain from rabid animals were incubated in blood plasma, and, second, to see if the characteristic bodies could be produced *in vitro* by combining normal brain and virus. We found that no development or multiplication took place *in vitro*, and that, altho structures indistinguishable from certain forms of Negri bodies developed in the virus-normal brain preparations, the same bodies were found in control preparations in which were pieces of normal brain without virus. In a single instance the virus remained alive after 8 days' incubation; animal inoculations of preparations incubated for a longer time gave in all cases negative results.

During the past year we have been applying Harrison's method to the cultivation and study of the virus of vaccinia. The results of our first series of studies appeared recently in this Journal.[3] We concluded that there was a definite multiplication of the virus in tissue cultures composed of blood plasma and pieces of cornea (of rabbit or guinea-pig) to which small quantities of diluted virus were added. Successful animal inoculations were obtained from the third subculture. Histological studies of the preparations,

---

*Received for publication November 3, 1913.

[1] Steinhardt, Poor, and Lambert, *Jour. Infect. Dis.*, 1912, 11, p. 459.

[2] Steinhardt, *Jour. Am. Med. Assn.*, 1913, 61, p. 1810.

Steinhardt, Israeli, and Lambert, *Jour. Infect. Dis.*, 1913, 13, p. 294.

however, revealed no specific vaccine bodies. The present paper is concerned with the continuation of these experiments.

Levaditi[1] has recently applied Harrison's method with some success to the cultivation of the virus of poliomyelitis.

We wish to record at this time experiments upon the following points: (1) estimation of the extent of multiplication of the virus in cultures by use of higher dilutions of original virus; (2) necessity of *living* corneal tissue for the growth of the virus; (3) possibility of substituting other organ tissues for cornea; (4) use of plasma and cornea from immune animals in the culture preparations.

USE OF HIGHER DILUTIONS OF VIRUS.

In previous experiments, described in our first paper,[2] the virus was diluted 1:5 before use in the cultures. Inoculation of a small number of unincubated preparations in the skin of rabbits gave in some instances 50–60 pustules; the incubated preparations, on the other hand, produced such extensive confluent eruptions that the number of pustules could not be estimated with accuracy. In order to determine the extent of multiplication of the virus, it therefore seemed desirable to make use of a higher dilution of the virus. With this end in view the following experiments were carried out:

Experiment 501.—Two commercial glycerinated viruses (2190 and 2193) were dialyzed in salt solution according to the method of Poor and Steinhardt[3] and placed in icebox to allow coarse particles to sediment. Only the supernatant fluid was used.

Virus 2190 was diluted 1:15 in Ringer's solution. A large number of preparations were made with rabbit cornea and plasma and the diluted virus, using the same technic as described in our first paper. Nine unincubated preparations inoculated in the skin of a rabbit gave 10 flat pustules (sixth day). It should be mentioned that with these preparations a very dilute virus gives frequently flat pustules. This may be due to a lytic action of plasma on virus. The same number of slides, incubated 11 days, gave upon inoculation 60–65 elevated pustules. Practically all of the preparations in this series showed contamination with bacteria, and, consequently, only a slight and transient growth of corneal epithelium. Whether or not bacteria interfere with the growth of the virus in tissue cultures, we are not yet prepared to say, since contaminations have been exceptional in our experiments. In this instance the virus was probably the source of the infection.

---

[1] *Compt. rend. Soc. de biol.*, 1913, 74, p. 1794.

[2] *Jour. Infect. Dis.*, 1913, 13, p. 294.

[3] *Jour. Infect. Dis.*, 1913, 12, p. 202.

Virus 2193 was diluted 1:15 in Ringer's solution and preparations made as before. Nine unincubated slides inoculated in the skin of a rabbit gave 6 flat pustules. Inoculation of the same number of preparations incubated 16 days (contaminated) produced 58 well-developed pustules.

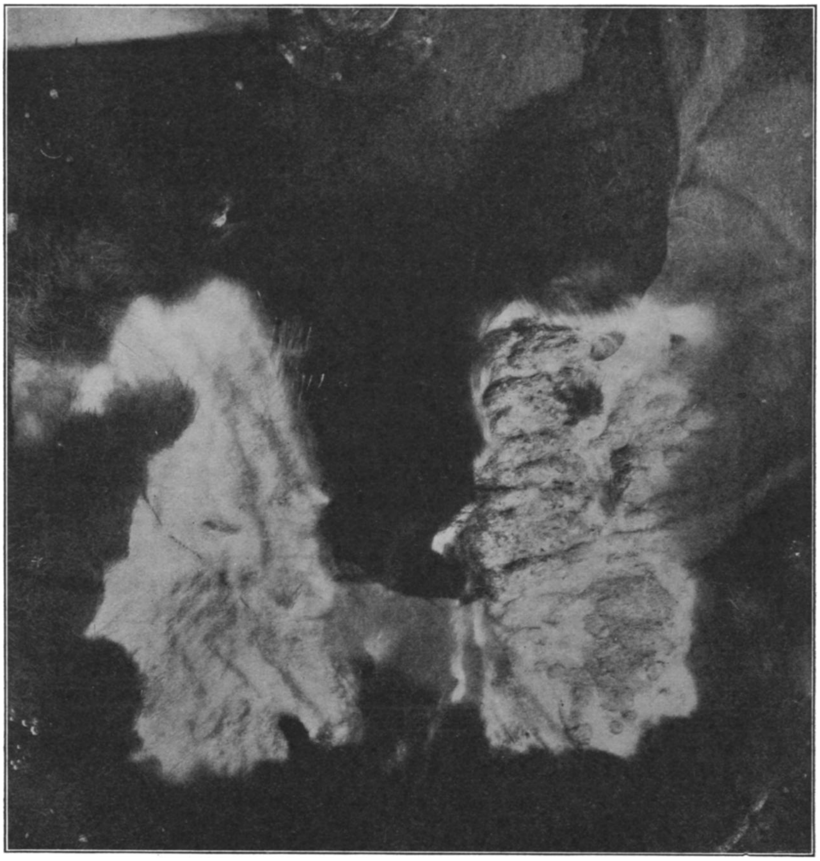

FIG. 1.—Skin of rabbit showing on its right side no "take," only scars from shaving, following inoculation of five "immune" preparations; and on left side, a confluent "take" from inoculation of control preparations.

These two experiments show, then, that in spite of bacterial contamination, which may conceivably interfere with the growth of the specific microorganism, the virus was increased six- to tenfold by incubation in the cornea plasma preparations. In several other experiments in which a lower dilution of the virus was used, the extent of growth appeared to be greater, but in no instance has the growth been comparable to that observed in cultures of rapidly growing bacteria. Irregularities in the rate of growth are to be expected, since they occur in the cultivation of practically all of the known microorganisms.

The method used here for estimating the degree of multiplication has been shown by Calmette and Guerin[1] to be reasonably accurate, comparing favorably with the method of plating for bacteria; it is in general use in commercial laboratories.

NECESSITY OF LIVING TISSUE FOR GROWTH OF VIRUS.

We have shown (first paper)[2] that when pieces of paraffin are substituted for cornea in plasma preparations, no growth of the virus takes place. To determine whether or not *living* cornea was necessary, the following experiments were carried out:

Experiment 503.—Virus 2186. Diluted 1:5 in Ringer's solution. The pieces of cornea to be used were divided into two lots; those in the first lot were frozen in an ice-salt mixture at -18° C. for 30 minutes, and were then used for virus cultures. The unfrozen tissue was used in control preparations. The frozen tissue upon incubation showed no outgrowth of either epithelium or connective tissue cells. Nine of these preparations incubated 12 days gave upon inoculation only 12–13 pustules; the same number of control preparations gave a confluent "take" composed of at least 300 pustules.

Experiment 504.—Virus 2186. Diluted 1:5. Pieces of cornea were placed for 45 minutes in weak hypotonic salt solution before being used. Inoculation of 9 unincubated preparations gave 50–60 pustules. Nine slides incubated 10 days were negative upon inoculation. There was also no "take" following the inoculation of 5 slides incubated 21 days. Microscopic examination showed that there was no growth of corneal tissue in any of the preparations. A second experiment was made at the same time using cornea killed by freezing as in Experiment 503. The results were practically identical with those just recorded for cornea killed by hypotonic salt solution.

These three experiments indicate that the vaccine virus does not grow in preparations containing corneal tissue killed by freezing or by a weak hypotonic salt solution. These two methods of destroying the vitality of the tissue were used because the chemical changes which they bring about are very slight. The results obtained are in harmony with the general conception of the close association of the virus with living body cells.

---

[1] *Ann. de l'Inst. Pasteur*, 1905, 19, p. 317.

[2] *Op. cit.*

SUBSTITUTION OF OTHER TISSUES FOR CORNEA.

Pieces of heart, liver, and kidney were used. Since the technic employed was the same as with cornea, it seems unnecessary to describe the experiments in detail. The results were as follows: The virus died out quickly in the liver preparations. This was due no doubt to the action of bile, which has been shown to be highly toxic for the virus. There was no evidence of multiplication in the heart and kidney preparations; on the contrary, there seemed to be a gradual weakening of the virus. Preparations

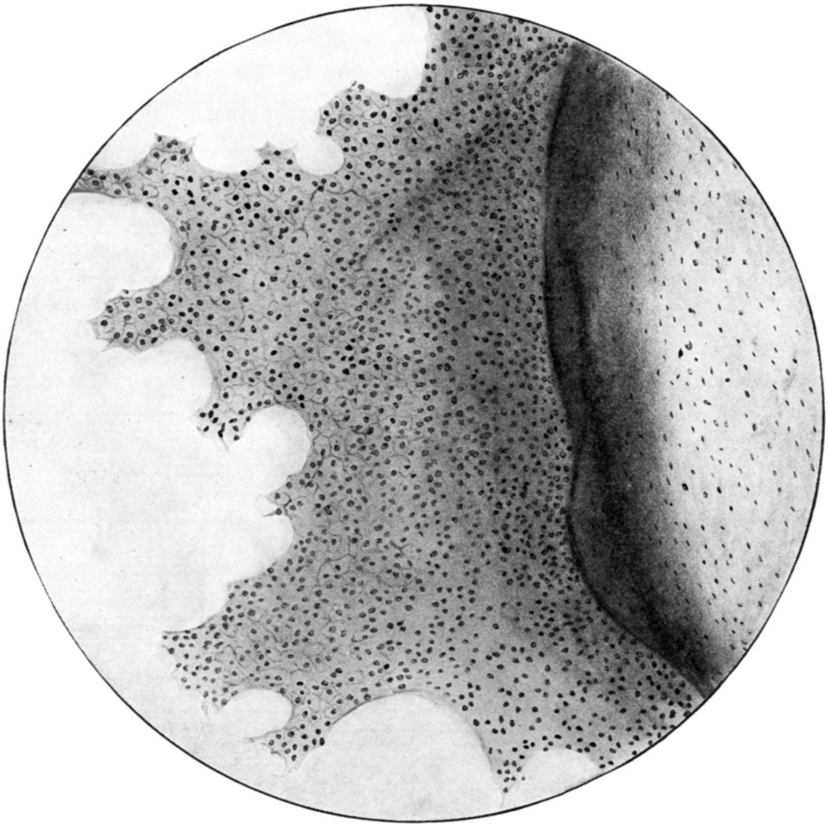

FIG. 2.—Tissue culture preparation of cornea showing outgrowth of a sheet of epithelial cells. Along the margin the sheet is only one cell thick, and the cell boundaries are quite distinct. The compact corneal fragment contains small oblong nuclei.

incubated for more than 10 days were found to be practically inactive.

USE OF PLASMA AND CORNEA FROM IMMUNE ANIMALS.

Immunity was established in a rabbit by a cutaneous inoculation of virus, resulting in an extensive eruption. Two weeks later the animal was bled, and the cornea removed for use in the following experiment.

Experiment 486.—Virus diluted 1:5. Two series of preparations were made: one, with plasma and cornea from an immune animal; the other, with plasma and cornea from a normal rabbit (controls). Inoculation of 5 "immune" preparations incubated 5 days gave a completely negative result (skin of rabbit, right side). Inoculation of same rabbit simultaneously on left side with an equal number of control preparations yielded a very extensive confluent "take." Repetition of this experiment a week later gave a similar result. In both experiments there were excellent growths of corneal epithelium.

This experiment shows that the virus becomes inactive in a short time in preparations containing plasma and cornea from an immune animal. The experiment also affords good proof of the adaptability of the method of tissue cultivation for the demonstration of many immunity reactions *in vitro*.

CONCLUSIONS.

1. The virus of vaccinia incubated in tissue cultures composed of plasma and cornea from normal rabbits and guinea-pigs shows a definite increase, but the degree of multiplication is not comparable to that observed in cultures of rapidly growing bacteria.
2. There is no growth of the virus in preparations containing cornea killed by freezing or by hypotonic salt solution.
3. There is no evidence of the growth of the virus in preparations in which pieces of heart, liver, or kidney have been substituted for cornea.
4. The virus is soon rendered inactive in preparations containing plasma and cornea obtained from an immune animal. The method of tissue cultivation is well adapted for the demonstration of immunity reactions *in vitro*.